OXYGEN CONSUMPTION OF THE MOLLUSC *POTAMOPYRGUS ANTIPODARUM* IN RELATION TO HABITAT

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ABSTRACT

Rates of oxygen consumption of the gastropod *Potamopyrgus antipodarum* from four different habitats; a polluted river, a non-polluted river, a still pond, and a brackish water lake, were determined and compared, and the influence of certain external factors examined. Snails living in still water had a lower metabolic rate than those living in running water, while those from brackish water had a significantly higher metabolic rate than those from fresh water. The rate of oxygen consumption of *P. antipodarum* from all areas remained fairly constant, despite decreasing oxygen tensions down to at least 5 mg/l. The rate of respiration increased with increasing temperature, but at a decreasing rate. Snails from both fresh and brackish waters increased their rate of oxygen consumption at higher salinities.

INTRODUCTION

Several laboratory studies have shown that the metabolic rates of certain freshwater invertebrates may differ in different habitats (Fox and Simmonds 1933; Walshe 1948; Mann 1961).

The hydrobiid gastropod *P. antipodarum* (Gray 1843) has colonized a wide range of habitats, being found in estuaries and brackish water pools as well as freshwater lakes and streams. This paper describes a laboratory study designed to determine whether there are differences in the respiratory rates of *P. antipodarum* taken from different habitats and to examine the influence of external factors, in particular temperature, oxygen concentration and salinity. The rate of oxygen consumption, determined under various experimental conditions, also provides an index of the metabolic capacity of the animals from each of the selected areas to withstand environmental stress.

A comparison between New Zealand's endemic *P. antipodarum* and the European species *Potamopyrgus jenkinsi* has shown that the two are anatomically identical and that many features of their biology and ecology are similar (Winterbourn 1972). It is therefore of interest to compare the respiration of these two species.

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SOURCES OF MATERIAL

Animals used in this study were collected from four distinct habitats in and around Christchurch, in the South Island, New Zealand. Approximate grid references are given.

HEATHCOTE RIVER (172°38'E, 43°34'S)

P. antipodarum was collected from the non-polluted upper reaches of the river, where the stony substrate was covered with an abundant growth of the macrophytes *Elodea canadensis* and *Myriophyllum* sp.

KAIAPOI RIVER (172°39'E, 43°23'S)

Snails were collected immediately below the effluent outlet of the North Canterbury Wool and Fellmongery Limited, where the gravel bed was coated with mud and soft deposits derived in part from the fellmongery. Near the north bank of the river, a small bed of *E. canadensis* was heavily populated with *P. antipodarum*. Water at this collection site may be completely deoxygenated at certain times during the day (Winterbourn *et al.* 1971).

LAKE ELLESMERE (172°39'E, 43°47'S)

Lake Ellesmere is a large body of brackish water separated from the sea by a permeable shingle bar, Kaitorete Spit. Fresh water flows into the lake from several streams and rivers, and from a number of artesian springs upwelling in the bed of the lake. The lake is opened at the southern end of the spit when necessary, to prevent its level from reaching an undesirable height. Therefore, salinity within the lake varies, depending on proximity to rivers and according to the opening of the lake. Salinities of 56% sea water have been recorded, but it is generally about 20% sea water (7‰).

Collections were made along the north-eastern margin of the lake. Here *P. antipodarum* was found in the mud, under stones, and on the leaves and triangular stems of the sedge, *Scirpus americanus*, which forms a narrow fringe along the lake edge.

ISAAC'S POND (172°32'E, 43°28'S)

This man-made, shingle based pond has a maximum depth of 4 m, and although only two years old, has a dense growth of *E. canadensis* covering its stony bottom. Large numbers of *P. antipodarum* were found within the macrophyte beds.

METHODS

COLLECTION OF ANIMALS

Large numbers of *P. antipodarum* were collected by sweeping a long handled dip net (mesh size approximately 1 mm square) through vegetation. They were transported to the laboratory in a large plastic bucket containing water from the locality. In the laboratory snails were transferred to a transparent plastic aquarium containing artificially aerated tap water (15°C ± 1°C) and small pieces of *E. canadensis* or, in the case of brackish

water snails, approximately 15% sea water and some fragments of *S. americanus*. The maximum time spent in these conditions in the laboratory was 48 h.

OXYGEN DETERMINATIONS

Oxygen determinations were made using a 5 ml glass syringe, according to Efford's (unpublished 1968) modification of the micro-Winkler technique. The precipitate was dissolved with phosphoric acid. A 10 ml burette, accurate to 0.01 ml, was used to titrate the contents of the syringe with 0.0025 N sodium thiosulphate.

SALINITY DETERMINATIONS

Salinities were determined by titration with silver nitrate in the presence of potassium chromate as indicator (Cox 1967).

DETERMINATION OF RESPIRATORY RATE

Experiments were carried out in McCartney bottles of known volume (29.1-30.1 ml), 30 snails of similar size being placed in each. The respiratory bottles were filled with water of the desired oxygen concentration from a 2-3 litre stock bottle using a "reversed bicycle pump" similar to that described by Mackereth (1963). At least three times its volume of water was passed through each respiratory bottle before it was carefully sealed with a screw cap, ensuring that no air bubbles were trapped. The range of oxygen values obtained in a test series of five 30 ml bottles filled in this way was 7.90-8.26 mg/l. Unless otherwise stated, artesian bore water was used in all experiments involving freshwater snails, while snails from brackish water were tested in water of 5‰ salinity.

For each set of measurements, three initial control bottles, three bottles containing snails, and one final control bottle were washed and filled with water. The initial control bottles were titrated immediately, while the others were placed in a dark, temperature-controlled room at 12.5°C. The darkness prevented photosynthesis of algae attached to the shells of snails. Unless otherwise stated, all experiments were run over a 1 h period, at the end of which all bottles were shaken and their oxygen content determined. The difference between the oxygen content of the initial control bottles and that of the bottles containing snails gave the oxygen uptake over that period. The change in oxygen tension between the initial and final control bottles enabled respiration by bacteria to be accounted for. This value was subtracted where necessary.

If air bubbles became trapped in any of the respiratory bottles or in the syringe during the experiments, then a new set of measurements was made.

After each experiment, all animals were placed in dilute hydrochloric acid to decalcify their shells. Soft tissue was then blotted dry and weighed. Oxygen consumption of the snails was related to blotted tissue (live) weight and expressed as (mg/g)/h. As snails were not narcotized during experiments, the respiratory rates obtained provide a measure of their active metabolism. It is probable that differences in activity account

for the rather large standard deviations (up to 20% of the mean) obtained in some cases.

STATISTICAL TESTS

Much of the data was analysed at the 0.05 level of confidence, using the standard Student's "t" test (Sokal and Rohlf 1969). Multiple comparisons were made using the Kruskal Wallis test (H statistic).

PRELIMINARY EXPERIMENTAL STUDIES

Preliminary investigations were made to assess the effects of certain factors associated with the methods employed.

RATE OF OXYGEN CONSUMPTION WITH INCREASING TIME

When the closed bottle technique for measuring the rate of oxygen consumption of a given species is used, an important initial factor to be decided is the length of time over which experiments are to be run. Conditions in the respiratory bottles change with time as the oxygen concentration decreases and waste metabolites accumulate. Using similar sized snails from the Heathcote River, a series of experiments was set up in which the amount of oxygen consumed during various time intervals was measured. The results of oxygen determinations made after 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7 and 8 h are shown in Fig. 1.

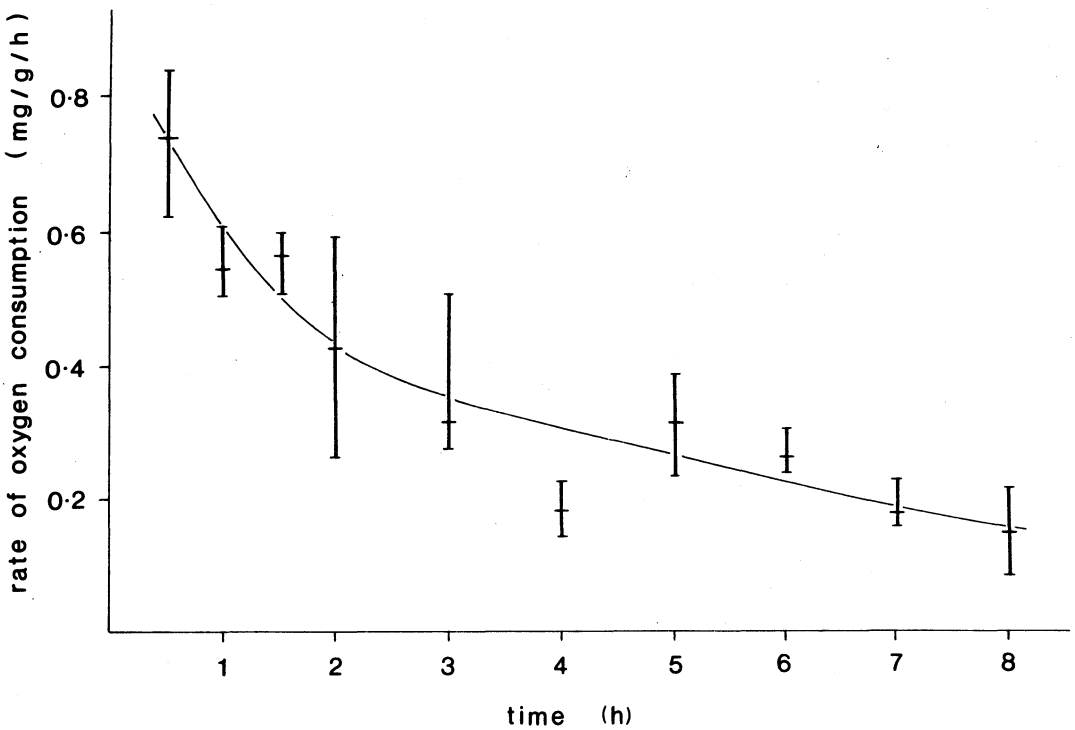

Fig. 1. Rate of oxygen consumption of snails from the Heathcote River with increasing time. Horizontal lines indicate means; vertical bars indicate range of 3-5 determinations.

A marked decline in the rate of oxygen consumption was found as the length of the experiment increased. This decline became more gradual with time. Repetition of this experiment with snails from the three other localities produced similar results. This decline was not due to starvation, as measurements of the rate of oxygen consumption of starved individuals made at intervals over a 10 h period showed no significant change ($H = 2.69$ for 7 degrees of freedom).

It seems likely that the heightened metabolism observed during the first 1-2 h is attributable to stress, the animals reacting to being transferred with forceps from one container to another, and they probably require time to adapt to the somewhat unnatural conditions of a "sterile" 30 ml glass bottle. After 3 h, the decline in respiratory rate became more gradual, but by this stage respiration may have been influenced by the fall in oxygen concentration in the bottle and possibly by the accumulation of metabolites.

Clearly, when using the closed bottle technique for comparative studies, it is imperative that a constant time interval be employed. In this case it was decided to run all further experiments for 1 h.

EFFECT OF SIZE ON OXYGEN CONSUMPTION

Oxygen consumption rates were determined for three different size classes of *P. antipodarum* taken from the Heathcote River. Results are shown in Table 1. Different sized snails had significantly different respiratory rates (Table 1), which were highest in the smallest snails. All subsequent experiments were carried out with snails in the modal size class, i.e. 2-4 mm.

TABLE 1. EFFECT OF SIZE ON RESPIRATORY RATE OF *P. ANTIPODARUM* FROM THE HEATHCOTE RIVER

Size class (shell height)	Mean respiratory rate (mg/g)/h ± S (n)
< 2 mm	0.760 ± 0.040 (4)
2-4 mm	0.544 ± 0.055 (4)
> 4 mm	0.418 ± 0.050 (3)

EFFECT OF AQUARIUM CONDITIONS

After collection, animals were brought back to the laboratory and placed in a large transparent aquarium in which they were kept for a maximum of 48 h. As conditions differed from those of the natural habitat, they could have affected the rate of oxygen consumption. This was tested by running a series of experiments with animals from the Heathcote River after they had been kept in the aquarium for various time intervals up to 48 h. No significant differences were found ($H = 4.356$ for 6 degrees of freedom).

EFFECT OF STILL WATER ON RESPIRATORY RATE

In completely still water, removal of oxygen from the surrounding water by respiration results in the formation of an oxygen depleted layer around the body of an animal (Kamler 1969). Since animals renew these boundary layers by an increase in

activity, it is possible that values for the rate of oxygen consumption, measured in a closed bottle in the complete absence of water movement, will be higher than rates occurring in the field. Experiments in which the respiration bottles were shaken at 10 minute intervals over a 1 h period were carried out. Respiration rates were slightly lower in the shaken respiration bottles but the differences were not statistically significant. In all further experiments the respiration bottles were left undisturbed.

RESPIRATORY RATES OF *P. ANTIPODARUM* FROM DIFFERENT LOCALITIES

The rates of oxygen consumption of *P. antipodarum* from the four localities were measured under similar initial oxygen concentrations. Results are shown in Table 2. Results of "t" tests indicated that at the 5% level of confidence, there were significant differences in respiratory rates between snails from all pairs of localities except those from the Kaiapoi and Heathcote Rivers.

TABLE 2. RESPIRATION RATE OF *P. ANTIPODARUM* FROM FOUR LOCALITIES

Locality	Initial oxygen conc. (mg/l)	Mean respiratory rate (mg/g)/h ± S (n)
Heathcote River	7.88	0.537 ± 0.046 (3)
Kaiapoi River	7.36	0.517 ± 0.059 (4)
Lake Ellesmere	7.09	0.796 ± 0.132 (3)
Isaac's Pond	8.11	0.382 ± 0.057 (3)

P. antipodarum taken from still water had a significantly lower rate of respiration than snails from running waters. This is in accordance with the generalization that still water forms, exposed to a slower rate of oxygen replacement in their immediate vicinity, have a lower respiration rate than their stream-dwelling counterparts (Hynes 1970). The most interesting feature of this comparison was the much greater oxygen consumption found in snails from the brackish water population. As brackish water animals are more nearly in osmotic equilibrium with their external environment, one might expect that their respiratory rate would be less, since less energy would be expended maintaining an osmotic gradient. The results obtained here contradict this hypothesis.

Possible explanations for the higher metabolic rates found in saline water will be discussed more fully later in this paper.

EFFECT OF TEMPERATURE ON RESPIRATORY RATE

The rate of oxygen consumption of snails from the four localities was measured at various temperatures between 4° and 28°C. Snails were acclimated for approximately 2 h to each experimental temperature before being placed in a respiration bottle at that temperature for 1 h. Results are shown in Fig. 2.

Water temperatures at the four localities were similar at the time of collection, and in the laboratory all populations were kept at the same temperature (15°C ± 1°C). Since all four populations had similar, recent thermal histories, it is not surprising that there was little difference in their responses

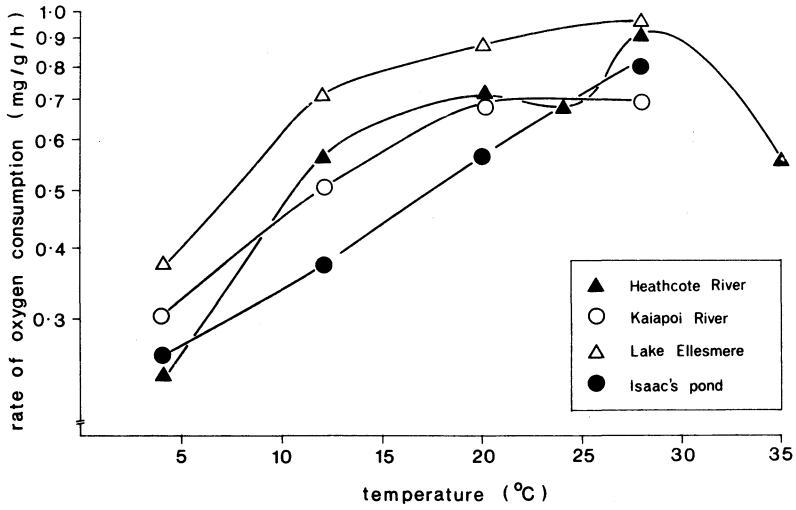

Fig. 2. Effect of temperature on the respiratory rate of *P. antipodarum* from four populations. Points given are the means of 3-5 determinations.

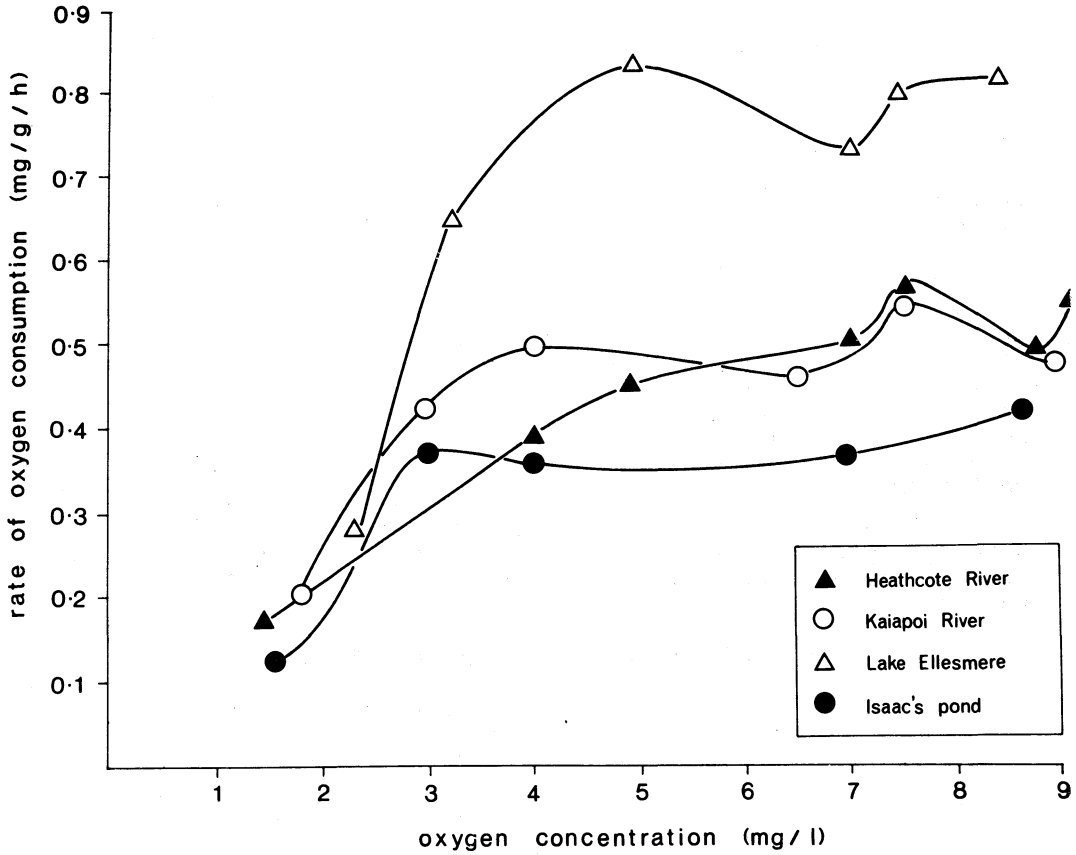

Fig. 3. Rates of oxygen consumption of *P. antipodarum* from four populations in relation to oxygen content of water.

to increasing temperatures. In each case the respiration rate increased with an increase in temperature, and there was no significant difference in the maximum respiratory rate of *P. antipodarum* from the four populations (H = 4.39 for 3 degrees of freedom). However, in accordance with Krogh's curve (Krogh 1916), this increase did not continue indefinitely. Beyond a certain point there was only a slight increase which, in the case of snails from the Heathcote River, was followed by a definite irreversible drop indicating adverse conditions. Presumably snails from the other three populations behave in a similar manner, although this was not examined.

The maximum respiratory rate of snails from the Heathcote River occurred at 28°C, which is the maximum temperature at which *P. antipodarum* has been found in the field (Winterbourn 1969).

OXYGEN CONSUMPTION AT VARIOUS OXYGEN CONCENTRATIONS

A series of experiments was designed to determine how *P. antipodarum* responded to different levels of dissolved oxygen, and whether this response differed in snails from different environments.

The oxygen content of the water was altered to the desired concentration either by bubbling air through the water to increase the oxygen concentration, or by heating it to reduce the oxygen level. Animals were taken from the aquarium and immediately placed in water of the experimental oxygen concentration at 12.5°C, i.e. they were in no way acclimated to the test conditions. The rate of oxygen consumption for each group was determined over the range 1.40-9.02 mg/l.

Snails from all four populations of *P. antipodarum* maintained a more or less constant rate of oxygen consumption so long as the oxygen concentration at the start of the experiment exceeded 5 mg/l (Fig. 3). The ability of *P. antipodarum* from still water to maintain a lower but constant rate of oxygen consumption, even at oxygen concentrations as low as 3 mg/l, is perhaps a useful adaptation to life in an environment in which, there is no rapid replacement of oxygen in the water immediately surrounding the animal. Similarly, the slightly lower incipient limiting point of individuals from the Kaiapoi River may reflect physiological acclimation to the low oxygen concentrations which occur frequently in this area as a result of local pollution.

This ability to maintain a constant rate of respiration despite declining oxygen tensions (as low as 3-5 mg O_2/l) helps explain why *P. antipodarum* is tolerant of mildly polluted waters.

SURVIVAL UNDER LOW OXYGEN CONDITIONS

A study was carried out to determine how long snails from the four localities could survive under low oxygen conditions. Approximately 40 individuals from each area were placed in sealed 30 ml bottles which were filled with water with an initial oxygen concentration between 1.00 and 1.75 mg/l. At intervals, the bottles were opened, the animals placed in oxygenated water (7.0-8.3 mg/l) from their natural habitat for 1 h, and the percentage of snails surviving determined. An animal was

considered dead if it showed no sign of movement during this period in oxygenated water.

Results are shown in Fig. 4. In all cases, oxygen levels determined after 50% of the population had died ranged from 0-0.5 mg/l. An interesting feature of this experiment was that at the time of death the snails had not withdrawn into their shells as occurs when temperatures are raised (Winterbourn 1969), but remained fully extended.

Animals from running water seemed less able to withstand prolonged periods of very low oxygen tension than those from other habitats. Snails from the Kaiapoi River showed a more gradual increase in mortality over the first 50 h than those from the Heathcote River. This was probably the result of more permanent acclimation to low oxygen concentrations which frequently occur in the Kaiapoi River. The increased tolerance to low oxygen concentrations of animals from still water is not unexpected, since replacement of oxygen in the immediate vicinity of these animals is not nearly as rapid as in running waters. This, together with the fact that many snails remain at the bottom of ponds amongst dense clumps of weed, makes them liable to exposure to low concentrations of oxygen. No satisfactory explanation can be given as to why snails from brackish water were able to survive for such long periods under low oxygen tensions.

In a closed bottle experiment such as this, it is difficult to ascertain the exact cause of death. The most obvious reason is a lack of oxygen but no doubt poisoning by metabolites is an important contributing factor.

RESPIRATORY RATE IN RELATION TO SALINITY

Snails from both freshwater (Heathcote River) and brackish water (Lake Ellesmere) habitats were used to measure the rate of oxygen consumption in relation to salinity. Snails were not acclimated to experimental salinities. The results given in Fig. 5 show that for both groups of snails the rate of oxygen consumption increased with increasing salinity. Although the oxygen consumption rate of snails from brackish water was generally slightly lower than that of freshwater snails in water of 0-9‰ salinity, differences were not significant except at 5‰ salinity ($t_s = 6.03$). When freshwater snails were acclimated to this salinity prior to being tested, however, this difference was no longer significant.

For interest, measurements were also made of the respiratory rate at various salinities of *Potamopyrgus estuarinus* Winterbourn, 1971, a species which is confined to brackish water (Winterbourn, 1970). A number of *P. estuarinus* were collected from the exposed mudflats of the Avon-Heathcote Estuary, Christchurch, where they were found grouped alongside or under stones, rotting logs and other debris. As with *P. antipodarum*, respiration rate increased with increasing salinity, although the rate of increase was more gradual (Fig. 5).

As mentioned earlier, one might expect animals living in waters of low salinity to have higher metabolic rates than those in water of a high salinity, as a result of the need to actively osmoregulate. The opposite occurred in the above experiments.

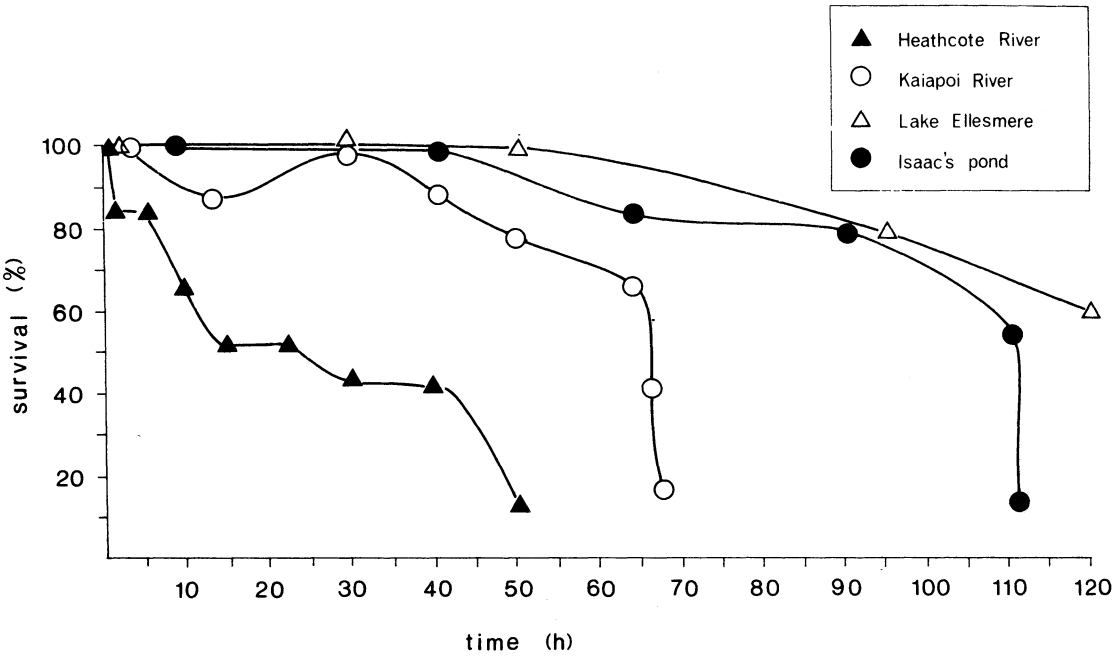

Fig. 4. Survival of snails from four populations at low oxygen tensions. Each point is the mean of 3 trials.

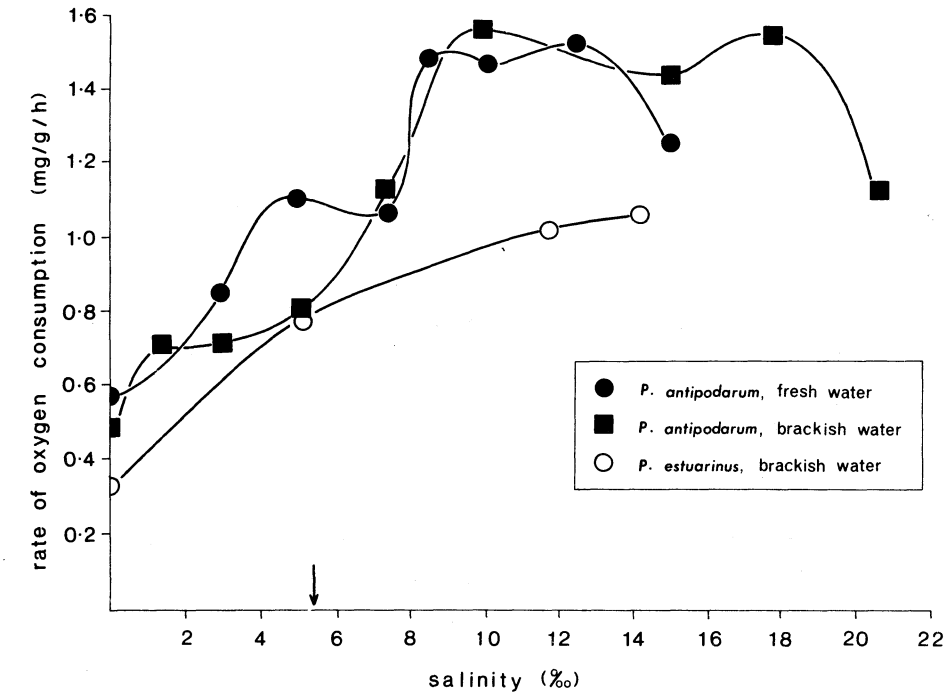

Fig. 5. Respiratory rates of snails taken from fresh and brackish water at different salinities. Points given are the means of 3-5 determinations. Vertical arrow indicates the habitat salinity of the brackish water population of *P. antipodarum*.

An adverse osmotic environment may also stimulate an animal to random movements or to escape movements (Potts and Parry 1964). Such an increase in activity would cause an increase in respiratory rate. When *P. antipodarum* was observed in water at a higher salinity to that to which they were accustomed, they appeared to be under some stress, as they frequently swivelled on one spot or displayed jerky movements. In a number of cases, particularly at higher salinities, snails seemed to "overbalance". Such stressed animals could also be expected to have an increased respiratory rate. If this were so, one would also expect *P. estuarinus* and *P. antipodarum* from brackish water to have a higher rate of respiration in fresh water (where they could be osmotically stressed) than in water of their natural habitat, rather than a lower rate as was observed.

Another possibility is that the change in metabolic rate with salinity may be related to the degree of hydration of tissues; i.e. any alteration of water content may influence the hormonal and enzymatic balance in the cells (Vernberg and Vernberg 1970). The extent to which this could affect the respiratory rate is not known.

COMPARISON WITH *POTAMOPYRGUS JENKINSI*

Because different techniques have been used in this and other studies to measure respiratory rates, it is not possible to make direct comparisons with values obtained for the European species, *P. jenkinsi*. However, it is interesting to compare trends in their responses to certain external factors.

In *P. jenkinsi*, oxygen consumption varies with temperature according to Krogh's curve (Lumbye 1958) and the results of this study indicate that *P. antipodarum* behaves in a similar manner.

P. antipodarum from all habitats examined was found to be capable of maintaining its rate of oxygen consumption down to at least 5 mg/l. Animals from polluted or still water, where fluctuating oxygen levels probably reach very low levels, had even lower incipient limiting points. At 16°C, the oxygen consumption rate of *P. jenkinsi* from fresh water has been found to fall off as soon as the oxygen content of the water decreases to approximately 20% (2 mg/l). In brackish waters, however, there is a tendency towards a slower falloff in oxygen consumption (Lumbye 1958). It must be remembered that in each case, the incipient limiting point is valid only for the particular environmental conditions under which it was determined, and it may change with various external and internal factors, e.g., temperature, activity, development stage of life cycle, size and acclimation to different oxygen tensions.

Lumbye (1958) showed that *P. jenkinsi* from brackish water had a greater oxygen uptake than snails from fresh water, but subsequently Lumbye and Lumbye (1965) found that the respiratory rates of *P. jenkinsi* from two freshwater localities were different, and that values for snails taken from brackish water were intermediate. In the studies of Lumbye (1958) and Lumbye and Lumbye (1965), all results were expressed as the rate of oxygen consumption per individual, and as the average size of animals collected from brackish water was greater than that of the freshwater group, the two results cannot easily be compared.

More standardized experiments have since shown that the respiratory response of *P. jenkinsi* to increasing salinities (Duncan 1966) is similar to that of *P. antipodarum* and also the New Zealand estuarine species, *P. estuarinus*. How changes in salinity act to produce this increased metabolism remains unresolved.

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